Taken together, we demonstrated activation of otherwise Ca^{2+}-dependent TRPM4 channels by U73122. These findings raise the possibility that endogenous substances act as physiologically relevant TRPM4 agonists.
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The Plasma Membrane TRPM8 Plays a Protective Role against Prostate Cancer Progression; *Trpm8* Gene as a Downstream Target of P53 Tumor-Suppressor
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Transient receptor potential melastatin 8 (TRPM8) is a cold-sensing and Ca^{2+} permeable channel. The mRNA of TRPM8 is overexpressed in early prostate tumors with high androgen levels, while anti-androgen therapy greatly reduces its expression. From the chromatin-immunoprecipitation (ChIP) analysis, we observed that an androgen response element (ARE) mediates androgen regulation of *trpm8*. Although TRPM8 mRNA is expressed at high levels, we found that the TRPM8 protein undergoes ubiquitination and degradation in prostate cancer (PC) cells. The mass-spectrometry analysis of TRPM8, immunoprecipitated from the membrane and total cell lysates of LNCaP cells identified ubiquitin-like modifier-activating enzyme 1 (UBA1), an enzyme that participates in the initial step of the ubiquitination cascade. We found that PYR-41, a potent inhibitor of UBA1, increased TRPM8 activity on the plasma membrane of LNCaP cells. Furthermore, PYR-41-mediated $_{PM}$TRPM8 activity was accompanied by enhanced activation of cell cycle inhibitor, p53 and apoptosis associated molecule, Caspase-9. In addition, we found that the promoter region of *trpm8* possesses putative binding sites for p53 and that the overexpression of p53 increased the TRPM8 mRNA levels in LNCaP cells. Our findings support previous studies that suggest the balance of androgen receptor (AR) and p53 expression regulates androgen-dependent growth of PC. Alternatively, effect of androgen / AR inhibition / TRPM8 overexpression on cancer cell proliferation and apoptosis was examined by FACS, TUNEL and clonogenic assay. All these experiments indicate that the TRPM8 protein stability is dependent on AR and could be negatively regulated at the post-translational level by AR. TRPM8 overexpression in PC cells showed anti-proliferative effect, suggesting the importance of TRPM8 channel as a tumor regulator.

1424-Pos Board B375
The Role of PLCδ4 in the Activity of TRPM8 Expressing Sensory Neurons
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Changes in environmental temperature are sensed by the peripheral endings of dorsal root ganglion (DRG) neurons. A subset of neurons expressing Transient Receptor Potential Melastatin 8 (TRPM8) channels function as major sensors of moderately cold temperatures. Previously it was demonstrated that Ca^{2+} influx through recombinant TRPM8 activates a Ca^{2+} sensitive phospholipase C (PLC) isoform, leading to depletion of phosphatidylinositol 4,5-bisphosphate with subsequent decrease of the TRPM8 currents (desensitization). We have found earlier that the most abundant highly Ca^{2+} sensitive PLC isoform in DRG neurons is PLCδ4. We performed patch-clamp experiments on TRPM8 expressing DRG neurons from PLCδ4-KO and WT animals, and identified 2 neuronal subpopulations based of their size. Small neurons had significantly larger TRPM8 current densities compared to the medium-large ones. PLCδ4-KO cells had larger currents upon menthol application and diminished desensitization. Current-clamp experiments further confirmed the differences in these subgroups. Both KO and WT small neurons had a more depolarized resting membrane potential than medium-large cells, and required smaller current injection to generate action potentials (AP) indicating their higher excitability. Positive current injection initially induced a train of AP during current pulses; increasing the current magnitude lead to a profound decline of the AP frequency in WT but KO neurons were able to generate AP at maximal frequency. AP shape and characteristics were also different for small and medium-large neurons. Negative current injection demonstrated characteristic voltage- and time- dependent rectification and rebound spike for both WT and KO small neurons. Menthol application induced transient generation of AP with frequency significantly higher in KO and larger depolarization compare to the WT neurons. Our present data support the role of PLCδ4 in the process of TRPM8 desensitization.

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A Novel Class of Transient Receptor Potential Melastatin 8 Agonists
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Transient receptor potential melastatin 8 (TRPM8) is a cold sensitive member of transient receptor potential ion channels. It is expressed by a subset of primary sensory neurons of the dorsal root and trigeminal ganglia and plays an important role in temperature sensation. Beyond cold temperature, several cooling agents, like menthol or icilin, also activate the channel. Here we report a chemically new class of agonists on the basis of macrocyclic lactone ring. In patch clamp and calcium imaging we found that the investigated compounds can activate the human recombinant TRPM8 overexpressed in HEK293T cells which activity was blocked by the TRPM8 antagonist N-(3-aminopropyl)-2-{[(3-methylphenyl) methyl]oxy}-N-(2-thienylmethyl)benzamide hydrochloride (AMTB). Some of the tested compounds were even more effective than the prototypic TRPM8 agonist menthol. Investigating various analogs, we identified a key motif of the compounds crucial in TRPM8 activation. Moreover, testing various mutants, we also identified amino acid residues forming the binding sites for these novel agonists on TRPM8. Investigating the native TRPM8 expressed by mouse sensory neurons we concluded that the most effective model compound is not only more potent but even more selective than menthol. Our results identified excellent chemical tools to study TRPM8 related functions and also highlight their potential pharmacological use.

1426-Pos Board B377
The N-Terminal Cleavage of PKD1L3 and its Effect on the Function of PKD1L3/TRPP3 Receptor/Ion Channel Complex
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Transient Receptor Potential Polycystin (TRPP) channels are a family of protein that forms functional receptor/ion channel complexes with Polycystic Kidney Disease (PKD) proteins that are involved in various physiological processes. For example, PKD1/TRPP2 complex have been known to be involved in renal physiology where a mutation in either of these proteins leads Autosomal Dominant Polycystic Kidney Disease (ADPKD). Another example is PKD1L3/TRPP3 complex, a sour taste receptor candidate, which response to low pH. PKD proteins share homology with adhesion G-protein coupled receptor proteins (aGPCRs). One of the key characteristics of these proteins is that they go through autoproteolysis at N-terminal GPCR proteolystic site (GPS), before the first transmembrane segment. The cleavage yield two fragments, one N terminus fragment (NTF) and another C terminus fragment (CTF). This autoproteolysis has been extensively studied in PKD1, however due to the lack of a method to activate PKD1/TRPP2 complex, the effect of the cleavage on the function of the channel is unknown. Since PKD1L3/TRPP3 can be activated with acid, it is a good model to study the effect of the cleavage on the channel function of this complex. Here, we show that cleavage happens on PKD1L3 at the GPS site as it is in PKD1, generating NTF and CTF. Mutations at the GPS site abolish cleavage. The non-cleavable mutant lost the ability to form functional channel with TRPP3, highlighting the significance of the cleavage in the overall function of the channel complex. Interestingly, co-expressed NTF and CTF not only associate with each other, but also form a functional channel in the presence of TRPP3 in Xenopus oocytes. Furthermore, we found natively non-cleavable PKD1L3 exits as a splicing variant, indicating potential function of the non-cleaved protein.

1427-Pos Board B378
Effects of Lipopolysaccharide on Sensory TRP Channels of Dorsal Root Ganglion Sensory Neurons
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Neurogenic inflammation and pain associated to bacterial infection have been ascribed to sensitization and activation of sensory nerve afferents. We have recently unveiled a role of Transient Receptor Potential (TRP) A1 channel as sensor of lipopolysaccharide (LPS) in nociceptive neurons. However, here we show that responses to LPS are still detected in 30% of dorsal root ganglion neurons isolated from Trpa1 KO mice. The proportion of cells responding to LPS was dramatically lower in double Trpa1/Trpv1 KO neurons. Using intracellular calcium imaging and patch-clamp in a recombinant expression system, we studied the effects of LPS on TRPV1, TRPV2, TRPM3 and TRPM8 heterologously expressed in HEK cells. In isolated sensory neurons, we compared the activation of TRPV1 and TRPA1 by LPS. In contrast, LPS was ineffective on TRPV2, weakly activated TRPM3 and activated TRPM8 at 25°C but not at 35°C. Our results indicate that, in addition to TRPA1, other TRP channels in